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Fungi and their mycotoxins in maize and maize silage

Storm IMLD, Sørensen JL, Rasmussen RR, and Thrane U

Introduction Maize silage is a major feed product for dairy cattle. A dairy cow can eat up to 40 kg a day. Unfortunately maize silage can be contaminated with mycotoxins both pre-harvest and post-harvest (Storm *et al.* 2008). This is potentially harmful to cattle, farmers and consumers of dairy and meat products. In cooperation between DTU Systems Biology, DTU Food, Aarhus University-Faculty of Agricultural Science, Danish Plant Directorate and Danish Cattle Association the extent of this problem has been examined and possible consequences evaluated.

Pre-harvest A screening of the presence of *Fusarium* in Danish maize revealed that the most common species was *F. avenaceum* (Fig. 1). This species produces moniliformin, enniatins and beauvericin, but none of the commonly surveyed *Fusarium* mycotoxins. A semi-selective medium was developed for isolation of *Alternaria* and *Phoma* (Sørensen *et al.* 2009) and used to survey maize samples collected at harvest. *Alternaria infectoria* was the predominant *Alternaria* species-group followed by *A. tenuissima* and *A. arborescens*, whereas *Phoma pomorum* was the only *Phoma* species isolated from maize.

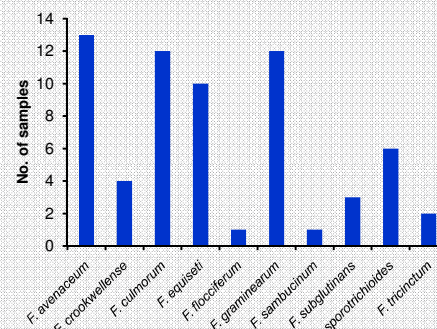


Fig. 1 Presence of *Fusarium* species in 28 maize samples collected at harvest 2005 and plated on the *Fusarium* selective medium Czapek-Dox Iprodion dichloran agar. The isolated *Fusarium* strains were grown on Yeast Extract Sucrose agar, potato dextrose agar and Spezieller Nährstoffarmer Agar and identified to species level based on morphology.

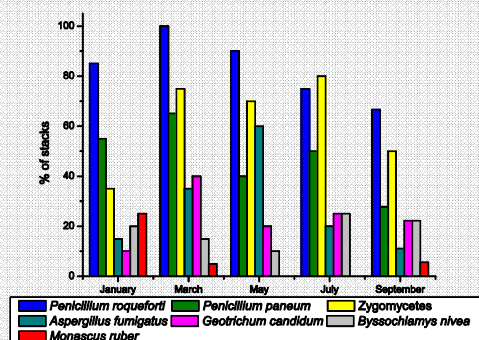


Fig. 2 The percentage of maize silage stacks from which specific filamentous fungi have been isolated in a whole-season study of silage mycobiota. 20 silage stacks were examined by repeated sampling over a 9 month period. Both core samples and hot-spots with visible fungal growth were collected and plated on V8-juice agar and dichloran glycerol 18% agar. All apparently different filamentous fungi were isolated on suitable media and identified according to Samson *et al.* (2002).

Post-harvest The species of filamentous fungi occurring in Danish maize silages and their frequency *in situ* was examined by monitoring 20 maize silage stacks over a whole season (Storm *et al.* in press). Viable propagules of filamentous fungi were present in all silage stacks at all times during the study. The most frequent species were *Penicillium roqueforti* and *Penicillium paneum*, *Zygomycetes*, and *Aspergillus fumigatus*. *Byssoschlamys nivea*/*Paecilomyces niveus*, *Monascus ruber* and *Geotrichum candidum* occurred less frequently. Yeasts and lactic acid bacteria were also enumerated but not identified. The average counts of all microbial parameters were shown to vary significantly over a storage season. The amounts of colony forming units of fungi were highest in 5-7 month old silage and significantly lower in 11 month old silage. The occurrence of hot-spots with visible fungal growth showed the same tendency.

Mycotoxins Methods for mycotoxin detection in maize and maize silage have been developed and validated for:

- Simultaneous extraction and analysis of 27 mycotoxins and other secondary metabolites from the most important toxigenic fungal species pre- and post-harvest. A simple pH buffered sample extraction was developed on the basis of a very fast and simple method for analysis of multiple pesticide residues known as QuEChERS and raw extracts analysed by LC-MS/MS (Rasmussen *et al.* 2010). The method was applied to 99 Danish fresh and ensiled maize silages (Table 1) and hot-spots of fungal growth.
- **Moniliformin** using hydrophilic interaction chromatography with UV and MS detection (Sørensen *et al.* 2007). Fifteen out of 28 analysed samples contained moniliformin at trace levels (<12 ppb)
- **Enniatins A, A₁, B and B₁** and the related compound **beauvericin** by LC-MS/MS. The method was applied to 76 maize samples (Sørensen *et al.* 2008).

Table 1 Mycotoxins and other secondary fungal metabolites detected in a survey of 99 samples of maize silage and fresh whole-crop maize. The samples were examined for 27 analytes by a rapid buffered extraction method followed by LC-MS/MS (Storm *et al.* submitted).

	Compound	n _{pos}	avg _{pos}	max
Quantitative	Alternariol monomethyl ether	3	8.8	11
	Andrastin A	15	169	691
	Alternariol	2	18	24
	Deoxynivalenol	7	1841	2974
	Enniatin B	28	75	365
	Mycophenolic acid	2	43	52
	Nivalenol	16	263	758
	Roquefortine C	2	173	189
	Zearalenone	34	71	666
	Citreosinocoumarin	8		
Qualitative	Marcfortine A	6		
	Marcfortine B	1		
	Roquefortine A	9		

Conclusion

- Mycotoxin producing fungi are ubiquitously present in maize and maize silage.
- In Denmark maize samples rarely contain any of the regulated *Fusarium* toxins in concentrations above the EU recommended levels for cattle feed.
- The observed occurrences of mycotoxins in 99 samples of maize and maize silage do not suggest that they are the cause of general problems observed at dairy cattle farms
- Fungal hot-spots in silage can contain substantial levels of e.g. PR-toxins, mycophenolic acid, citrinin and gliotoxin.
- More knowledge on long-term exposure and possible synergistic effects is needed.



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